THE EFFECT OF SOY AND FRUCTOOLIGOSACCHARIDES ON THE SELENIUM STATUS OF POSTMENOPAUSAL WOMEN

A Senior Thesis

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by

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Honors Project Abstract

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Title: The Effect of Soy and Fructooligosaccharides on the Selenium Status of Postmenopausal Women

This project focused on the nutritional status of postmenopausal women consuming soy. Specifically, this project examined the effect of soy and a prebiotic fiber source, fructooligosaccharide (FOS) on the status of the trace mineral selenium. Soy intake is of current interest to women's health because of its potential to decrease the risk of menopausal side effects, and chronic diseases such as heart disease and cancer. Soy's effects may be elucidated by the plant estrogens it contains. FOS is a prebiotic which can stimulate gut microflora, resulting in a possible increase in soy bioavailability. Soy products typically contain a significant amount of selenium and the soy phytoestrogens may also influence selenium status. Twenty-four postmenopausal women were recruited and were randomly assigned to the placebo (soy/no FOS) or a treatment (soy/FOS) group. During the treatment phase, each woman consumed a soy shake, and either FOS or a placebo powder. Blood plasma samples were collected during the baseline portion of the study (before soy/FOS or soy/no FOS treatment) and at the end of the two week treatment portion. These plasma samples were assayed for selenium concentration, and for the activity of the selenium containing enzyme, glutathione peroxidase (GPx). Selenium status was determined using gas chromatography, and GPx status was determined using a spectrophotometry. Four-day diet records were obtained from each subject at baseline and at the end of treatment and analyzed to determine an approximate daily selenium intake. The selenium in the soy supplement was determined to be bioavailable, as the placebo group had a significant increase ($P<0.05$) in plasma selenium. However, both the placebo group and the group receiving FOS had a significant decrease in GPx activity, ($P<0.014$) and ($P<0.01$) respectively. Thus, the isoflavones in the soy supplement or the FOS may have interfered with the bioefficacy of the selenium in the soy shake.

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Table of Contents

Introduction:

Soy intake is of current interest to women's health because of its potential to decrease the risk of menopausal side effects, and chronic diseases such as heart disease and cancer. As the popularity of soy supplementation increases, it is important to understand how soy is metabolized, as well as how it affects the metabolism, and bioefficacy, of other nutrients. Thus, the goal of this study was to determine the effect of soy and a probiotic fiber source, fructooligosaccharide (FOS), on the status of the trace mineral selenium. The plant estrogens, called isoflavones, present in soy may elucidate soy's beneficial effects. FOS is a prebiotic which can stimulate gut microflora, resulting in a possible increase in soy isoflavone bioavailability. Additionally, soy products typically contain a significant amount of selenium.

Soy is of interest relative to selenium status because of the apparent relationship between selenium and estrogen metabolism (1, 2, 3) and the fact that soy contains isoflavones, sometimes called phytoestrogens, that have a structure similar to that of estrogen (4). Selenium is a trace mineral that is a component of several selenoproteins including the glutathione peroxidases (GPx), which catalyze the reduction of harmful peroxides (5). Maintaining an optimum level of selenium and GPx, therefore, is important for protection against the development of diseases induced by oxidative damage such as cardiovascular disease and cancer (5). A relationship between selenium and estrogen status has been demonstrated in female rats (1) and women (2, 3). Since the structure of isoflavones and estrogen

are similar, it is hypothesized that selenium status will fluctuate with isoflavone levels in the blood. Consequently, if isoflavone absorption is enhanced by the addition of FOS, selenium levels may also increase with the addition of FOS to soy isoflavone supplementation.

Therefore, the objective of this study was to determine the effect of soy and fructooligosaccharides intake in postmenopausal women on the status of the trace mineral selenium, as measured by plasma selenium and glutathione peroxidase levels. We expected these measures of selenium status to increase from baseline to treatment due to the addition of soy isoflavone to the diet. We also expected an even greater increase in selenium status with the addition of FOS to the soy isoflavone supplement.

Literature Review:

Soy and Menopause

At the onset of menopause, many women experience unwanted symptoms and risks associated with a decrease in estrogen levels. Common symptoms associated with this decrease include hot flashes and weight gain. Also associated with this period is a more rapid loss in bone mineral density, increasing the prevalence of osteoporosis in the menopausal/postmenopausal population. More significantly, this population experiences an increase in the risk and incidence of coronary heart disease and cancer. In the past, hormone replacement therapy (HRT) has been used to reduce menopausal symptoms and risks. However, in 2002 a Women's Health Initiative study to determine the long-term effects of HRT was ended early due to negative side effects (6). Therefore, many women are exploring other options to relieve menopausal symptoms.

Some health care professionals recommend the addition of soy to the diet to prevent disease or reduce the severity of symptoms associated with menopause. Many studies have been conducted regarding the effect of soy products on the health problems of peri- and postmenopausal women. The results of one recent study suggest that the isoflavones in dietary soy impart a protective role against cardiovascular disease (CVD) in postmenopausal women (7). This study also found that women who regularly consumed a high amount of isoflavones benefited from a significantly lower waist circumference, body mass index, and fasting insulin levels. Potter et al (8) found that women who consumed 90 mg of soy isoflavones daily for

six months displayed a significant delay in the loss of bone mineral density, and showed a slight increase in the bone mineral content of the trabecular bone of the lumbar spine. Yet, the ability of soy to treat hot flashes remains controversial. Washburn et al (9) found that, compared to the placebo group, women who consumed 40 g of soy daily had a significant reduction in the severity of hot flashes. However, Murkies et al (10) found no significant difference in the frequency of hot flashes in menopausal women who consumed soy verses those who did not.

Soy Isoflavones and Phytoestrogens

Soy products contain isoflavones, sometimes called phytoestrogens, that have a structure very similar to that of estrogen (4). Soy is of interest relative to selenium status because there is an apparent relationship between selenium and estrogen metabolism (1, 2, 3). Thus, isoflavone intake from soy may have some effect on selenium status, and it is hypothesized that selenium status will fluctuate with isoflavone levels. The structure of naturally occurring isoflavones can inhibit them from being absorbed across the human intestinal enterocyte. Isoflavones are glycoside conjugates, thus it is hypothesized that removal of the glucose molecule by a glycosidase must convert the glycoside to an aglycone derivative before it can be absorbed. Genistin is a common isoflavone consumed by humans that must be converted to genistein, or dihydrogenistein, to be absorbed. This mechanism was demonstrated by Setchell et al (11), as they found that β-glucosidases are required for β-glucosides to be absorbed in the gut.

Fructooligosaccharides (FOS) are prebiotics, that is, they are a nondigestible food ingredient that are known to enhance bifidobacteria production in the colon (12). In 2000, Uehara et al (13) suggested that these gut microorganisms have glycosidase activity. Thus, it was hypothesized that by enhancing production of bifidobacteria FOS will increase the absorption of isoflavones in humans (13). However, in a more recent study, Nettleton et al (14) found that the consumption of a prebiotic/probiotic mixture did not enhance isoflavone metabolism in a group of postmenopausal women. In this crossover study, the women received a soy protein supplement, along with a small quantity of FOS (15-30 mg per day) and two probiotics, *Lactobacillus acidophilus* DDS and *Bifidobacterium longum* (14). There was no significant effect of the FOS/probiotic treatment on the plasma phytoestrogen concentration (14). Thus, further research is needed to determine the relationship between soy isoflavone absorption and FOS.

Selenium and Estrogen

A relationship between selenium and estrogen has been demonstrated in both the animal model and in humans. Smith, et al (1) showed that plasma selenium and GPx activity fluctuated during the rat estrus cycle and were significantly greater at the phase of the cycle when estrogen level is at its highest. Similarly, plasma selenium concentrations, and plasma and erythrocyte GPx activities, fluctuated in synchrony with estradiol throughout the menstrual cycle (2). Previous results also suggest that the decrease in estrogen that occurs at menopause may be associated with changes in selenium status in women since measurements of selenium status

of older post-menopausal women were lower than that of their perimenopausal/menopausal daughters (3). Thus, we hypothesize that measurements of selenium status will be positively correlated an increase in phytoestrogen intake from soy.

Important Roles of Selenium

Selenium is a trace element that is an essential component of several proteins including some antioxidant enzymes. The bioavailability of selenium in food products is usually high, and 50-90% of dietary selenium is usually utilized (15). The function of selenium is exerted by selenium containing proteins, called selenoproteins. More than 20 of these selenoproteins are known to exist (15). One example of an antioxidant selenoprotein is gluatathione peroxidase, which metabolizes toxic molecules, such as hydrogen peroxide and lipid hydroperoxide, from the blood (15). Therefore, a deficiency of selenium in the diet can lead to oxidative stress. The Recommended Dietary Allowance (RDA) for selenium is 55 µg per day, with an upper limit of 400 µg per day.

It has also been suggested that selenium can help prevent chronic diseases such as heart disease and cancer. Selenium has been associated with several heart protective functions. Selenium deficiency in the blood has been linked to an increased incidence of myocardial infarction and an increased death rate from cardiovascular disease (5). Selenoproteins help to breakdown fatty acid peroxides that can cause blood clots (5), and they also oxidize low-density lipoproteins (LDLs)

in blood vessels (5). Supplemental selenium has been associated with reductions in the incidence of lung, colorectal, and prostate cancers, as well as an overall incidence of cancer (5). These benefits of selenoproteins show the importance of maintaining adequate levels of the trace element in the blood, as well as demonstrate the importance of understanding supplemental selenium metabolism in relation to other dietary supplements.

Methodology:

Study Design. This study was part of a larger ongoing study on the effect of the fruit fiber, FOS, on soy isoflavone bioavailability in postmenopausal women. The original project, as well as the proposed project, were approved by The Ohio State University Institutional Review Board. The larger project was a placebo-controlled, single-blinded (with placebo powder instead of the FOS product) clinical study with a randomized block design. The control group (Treatment A) received a dextrose placebo powder as well as the soy protein supplement. The experimental group (Treatment B) received FOS powder as well as the soy protein supplement.

Subjects. This study included the 24 healthy postmenopausal women that were recruited for the original project. Postmenopausal was defined as not having a menstrual period within the 12 months before study initiation. Subjects could not be older than 70 years (inclusive) of age. They could not have taken any medication that would alter lipid, bone, or calcium metabolism, including hormone replacement therapy six months prior to study enrollment. The subjects had to be free of chronic disease and must have no history of gastrointestinal or malabsorptive disorders. The subjects could not be lactose intolerant. Additionally, the subjects could not have taken soy products or FOS products, antibiotics or probiotics the three months prior to study enrollment.

Treatment. Subjects participated in a five week protocol that was broken up into two periods. The Baseline Period lasted two weeks, during which subjects ate their

normal diet, except for any foods inherently high in soy or FOS. Subjects were then admitted to the General Clinical Research Center (GCRC) on the Wednesday of the second week of the Baseline Period. While in the GCRC, the subject fasted from 6 pm until 6 am on the following day. During the 20 hour stay, 4 blood samples and a partial 24 hour urine sample were obtained from the subject. The remainder of the urine sample was collected from the subject's home. Also during the Baseline Period the subjects were randomized to either Treatment A or Treatment B. The Treatment Period lasted three weeks.

Treatment A subjects consumed a Health Source ® Soy Protein Shake (Ross Products Division, Abbott Laboratories, Columbus, Ohio) every morning prepared with 2 scoops of shake powder and 8 fl oz of skim milk. A two scoop serving provided 26 g of soy protein and 80 mg of soy isoflavones. Treatment A subjects received a dextrose placebo powder (Sigma Chemical, St. Louis, MO) instead of the FOS. Treatment B subjects consumed a Health Source ® Soy Protein Shake and the Nutraflora ® Short chain Fructooligosaccharide FOS Powder (P95, Golden, Colorado, GTC Nutrition LCC). The FOS Powder was dosed incrementally into three evenly divided doses to develop subject tolerance. By the fourth day subjects received the maximum dose of 10 g of powder per day.

Subjects were counseled to remove foods high in soy or FOS from their diet for the duration of their five week participation in the study. (This did not include the treatment soy and FOS supplements.) They were also instructed on keeping their

protein intakes similar across study periods. In order to enhance counseling, subjects were asked to record their dietary intake for four days prior to the Baseline visit.

Sample Collection. Blood was collected for plasma and erythrocyte selenium and glutathione peroxidase analysis. Blood samples (3 mL) were drawn at 2 and 8 hours following ingestion of a soy product with known levels of isoflavones. Samples were drawn into evacuated tubes containing potassium EDTA. They were labeled with the subjects' code numbers and collection dates. They were then immediately placed on ice and then centrifuged (10 min, 4°C, 3000 X g) to separate the plasma and then will be stored at – 80°C until analysis (22). Plasma samples were analyzed for total selenium and glutathione peroxidase (GPX).

Dietary Intake. Subjects kept diet intake records for four (4) days of each of the 4 weeks preceding the GCRC admissions. Diet records were analyzed for selenium intake and other key nutrients using the ESHA Food Processor software, version 7.9 (2001, ESHA Research, Salem, Oregon).

Laboratory analyses. Selenium status was assessed based on measurements of plasma selenium and glutathione peroxidase (GPx) activity. Selenium concentrations were determined using the gas chromatography technique of McCarthy et al. (1981) using an Agilent 6890 Series gas chromatograph (GC) with a 225 Durabond Megabor column and an electron capture detector maintained at the

following temperatures: oven (column) 190^{o}C, front inlet (injector) 220^{o}C, and front detector 300^{o}C. Nitrogen (~60 psi) and helium (~80 psi) were used as the carrier gases. The data was integrated using an Agilent 6890 Series Integrator. Bovine liver (Standard Reference Material 1577b, National Institute of Standard & Technology, Gaithersburg, Maryland) was used as a reference standard.
Plasma GPx activity was determined by the method of Paglia and Valentine (1967) using a Shimadzu UV-visible recording spectrophotometer (model UV160U). Using this method, one milliunit of activity is equivalent to 1 nmol NADPH oxidized per minute. Total protein concentration was determined using the protein assay by Lowry et al. (1951) using the Shimadzu UV-visible recording spectrophotometer (model UV160U) at 500 nm.

Data Analysis. Statistical analyses were performed using SAS (Version 8.02, SAS Institute Inc., Cary, NC, 2001). Descriptive statistics (mean, standard deviation, median, range, percent change) were calculated for each of the variables of interest at each time point. MINITAB® (Version 14.13, Minitab Inc. 2004) was used to perform a paired student's t test to determine the significance of the data. The baseline data on selenium status from each subject prior to soy consumption allowed each subject to serve as her own control.

Results:

Dietary selenium intakes, shown in Figure 1, were determined from the four-day diet records obtained at baseline and at the end of the treatment period, and analyzed by Food Processor ® SQL. Dietary selenium intake tended ($P<0.06$) to decrease in the placebo group from baseline to treatment. However, dietary selenium intake did not change in the FOS group. The soy shake contributed 24 mg selenium to the total selenium intake per day. When taking this into account, there was no change in total selenium consumption for the placebo group. There was a significant increase in total selenium intake for the group receiving FOS ($P<0.02$).

The results of the subjects' plasma selenium data are found in Figure 2. The group that received the placebo powder showed a significant increase in plasma selenium ($P<0.05$) from baseline to treatment. The group receiving the FOS powder showed no significant change in plasma selenium from baseline to treatment.

The results of the subjects' plasma gultathione peroxidase (GPx) data are shown in Figure 3. GPx activity decreased significantly from baseline to treatment in both the placebo ($P<0.014$) and FOS ($P<0.01$) groups.

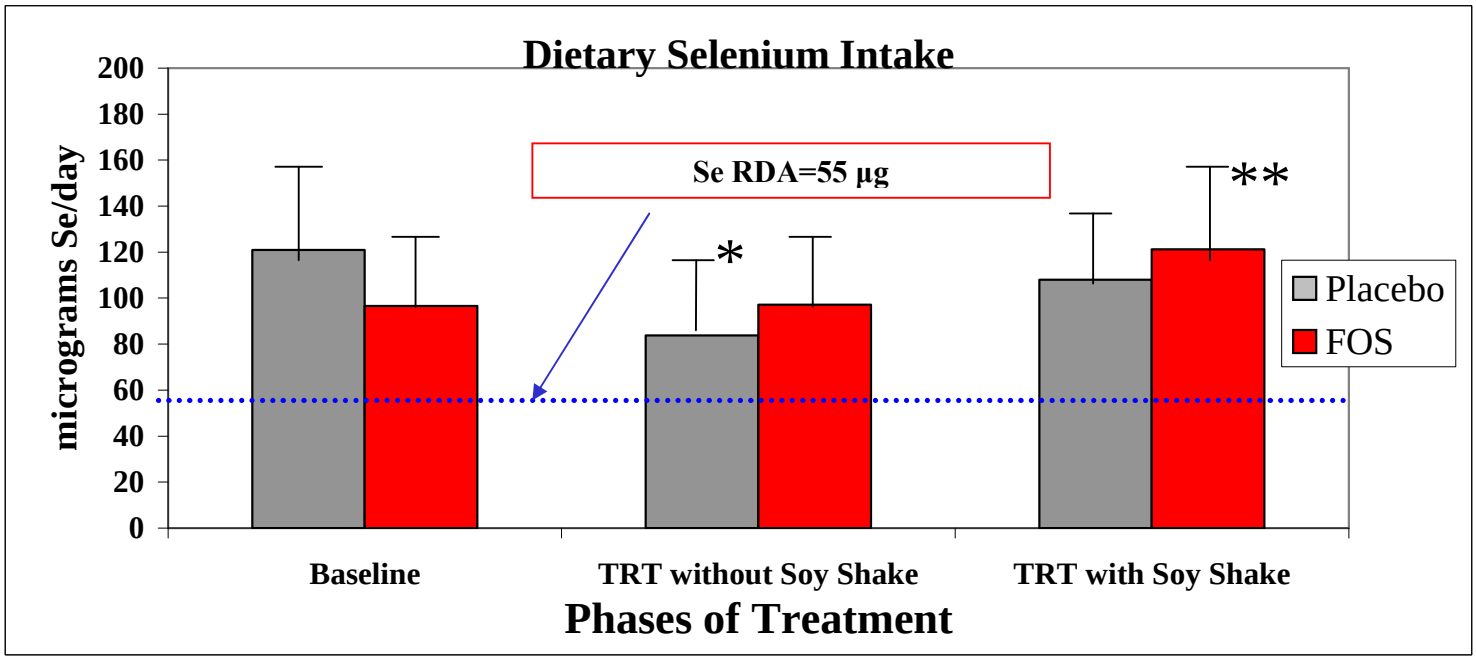

Figure 1. Dietary selenium intake at baseline and after treatment, with and without the soy shake. *Baseline<Treatment, P<0.06. **Baseline>Treatment, P<0.02

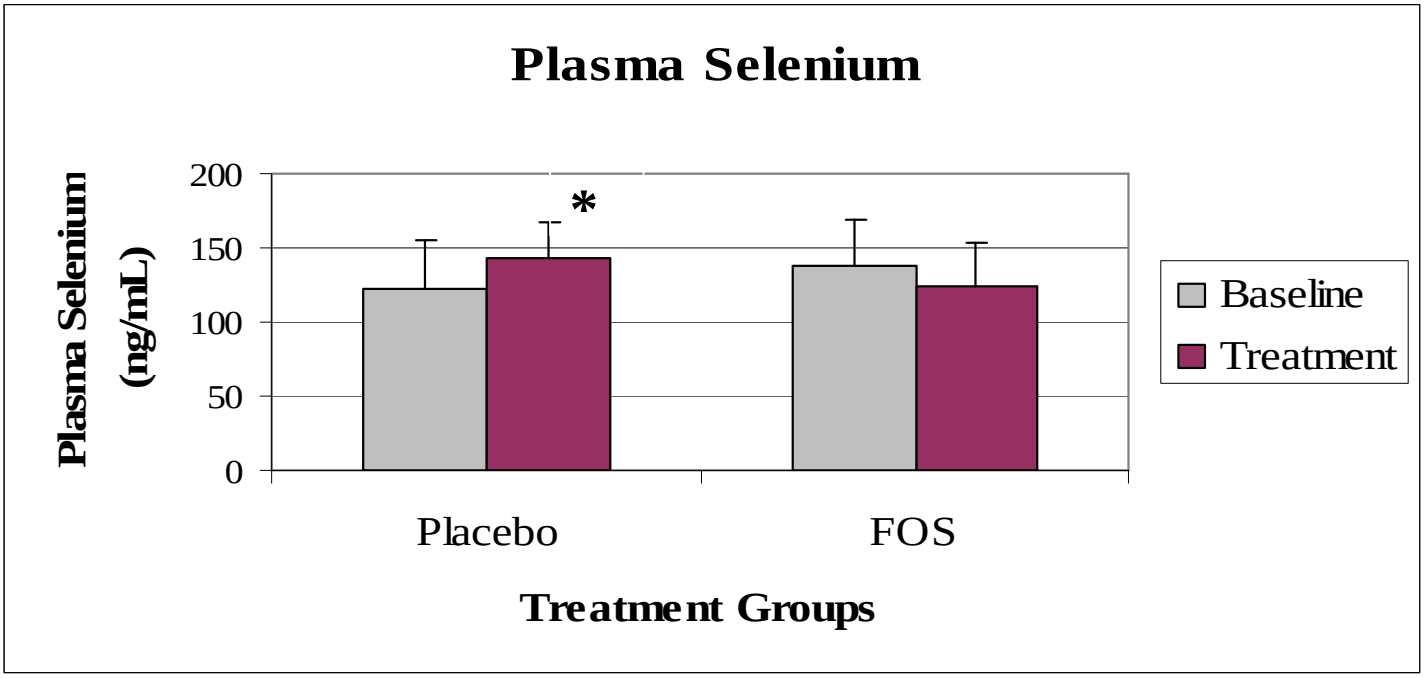

Figure 2. Plasma selenium concentration in postmenopausal women at baseline and after soy treatment with placebo or FOS. *Baseline<Treatment, $p<0.05$.

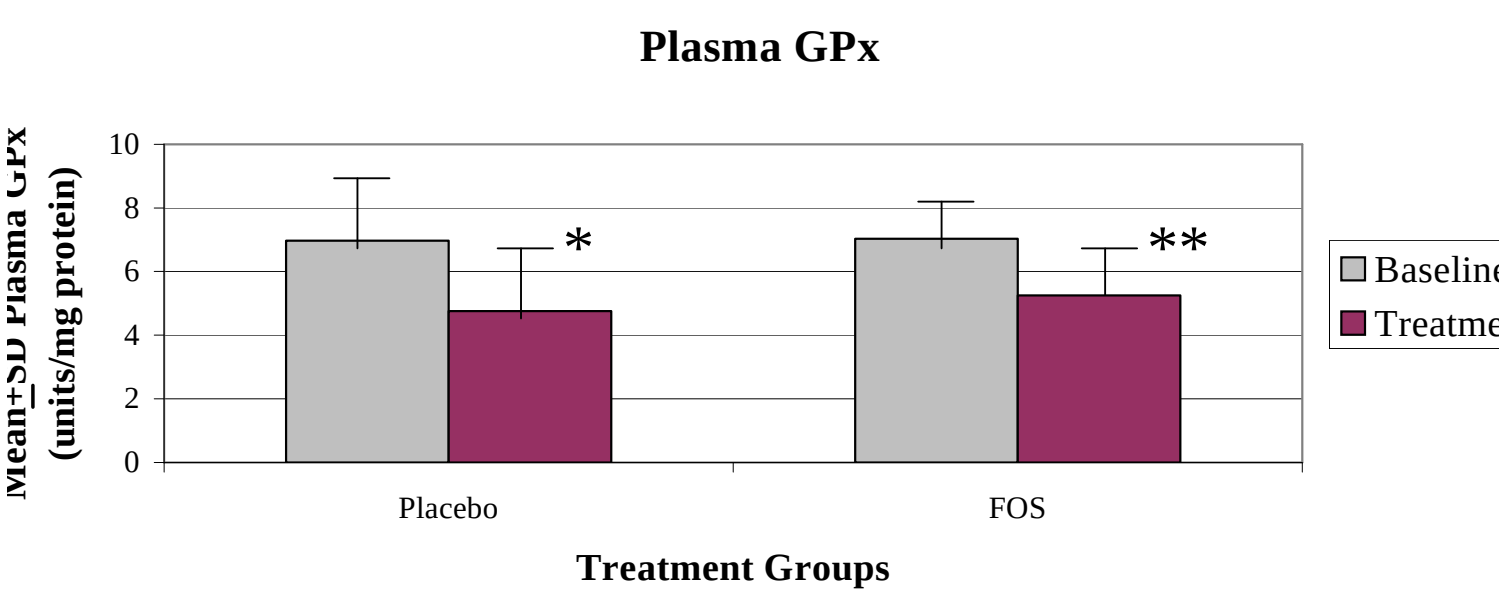

Figure 3. Plasma GPX activity in postmenopausal women at baseline and after soy treatment with placebo or FOS. *Baseline>Treatment, p<0.014, **Baseline>Treatment, p<0.01.

Discussion and Conclusions:

Plasma selenium concentrations increased with the consumption of a daily soy supplement, with no FOS, in the placebo group from baseline to treatment. The placebo group consumed an additional 24 µg of selenium per day from the soy supplement. However, since there was a trend ($P<0.06$) of decreased dietary selenium intake between baseline and treatment for this group without the soy powder, the group's total selenium intake did not change from baseline to treatment. These results suggest that the selenium in the soy powder was highly available since it increased the placebo group's plasma selenium levels from baseline to treatment. Although the total selenium intake of the FOS group increased significantly ($P<0.02$) with the addition of the soy supplement and FOS from baseline to treatment, this increase resulted in no significant change in the group's plasma selenium from baseline to treatment. These results suggest that the addition of FOS to the soy powder may have decreased the absorption of the selenium in the soy powder. In a recent study of short chain FOS (sc-FOS) in healthy postmenopausal women in France, selenium and zinc absorption were not affected by sc-FOS, where as copper absorption was enhanced (16). Stable isotopes of selenium, zinc and copper were used to measure mineral absorption in the French study (16), however, no measurements were made on blood parameters of the status of these minerals, making it difficult to compare to our results.

Although the selenium in the soy powder appeared to be bioavailable in terms of plasma selenium concentrations, the results GPx activity suggest that it may not have sufficient bioefficacy relative to the GPx activity. Bioefficacy is defined as the ability to change a functional parameter in the body. Low bioefficacy was suggested by a significant decrease in plasma GPx activity in both the placebo group ($P<0.014$) and the FOS group ($P<0.01$) between baseline and treatment. Thus, the selenium that was added to the diet did not result in an increase in GPx activity.

The consumption of soy isoflavones, thought to have estrogenic activity, therefore did not affect selenium metabolism as expected from previous findings on the relationship of estrogen and selenium in pre- (2) and postmenopausal (3) women. In premenopausal women, plasma selenium, plasma GPx activity, and erythrocyte GPx activity were greatest as estrogen levels peaked during the menstrual cycle (2). These results demonstrated that there was a linear relationship between these parameters of selenium status and levels of plasma estradiol throughout the menstrual cycle (2). Smith et al (3) also determined that there was a positive correlation between plasma selenium and plasma estrogen values over three generations of women. The results of this study also found that the group of postmenopausal grandmothers had the lowest plasma selenium levels when compared to their daughters (40-58 yr) and their granddaughters (19-26 yr) (3). Yet, our study did not find this increase in plasma GPx activity with the addition of soy isoflavones to the diet. Soy isoflavone absorption was assessed in the current study by measuring the increase in urinary isoflavone concentrations between baseline

and treatment. Results from the larger study indicate that urinary isoflavone concentration did increase as a result of soy and soy + FOS consumption (17).

To our knowledge, there have not been any previous studies that have examined the relationship between soy isoflavones, FOS, and selenium status. Although Ducros et al (16) evaluated the absorption of selenium relative to FOS consumption, no measurements of selenium status were taken. The addition of the soy to the diet appeared to increase selenium intake and plasma selenium but other factors in the soy, as well as the FOS, may have affected the ability of selenium to be incorporated into the functional proteins such as GPx. The effect of soy and FOS on the other parameters of selenium status, such as selenoprotein P, were not measured in this study. Perhaps as a result of the soy isoflavones, the soy selenium is being directed to, and incorporated into, another selenoprotein at the expense of GPx, or the isoflavones may be having an inhibitory effect of GPx production. Although previous studies have shown a relationship between selenium and estrogen in women (2,3), soy phytoestrogens may not have similar estrogenic effects on measures of selenium status and the production of selenoproteins. The results of a study of healthy postmenopausal women in Australia support this hypothesis in that, biological indicators of estrogenicity, including hepatic protein synthesis and gonadotropin concentrations, were not affected by dietary soy isoflavone consumption (18).

It is hard to predict the biological effect of phytoestrogens since they have been shown to act as estrogen agonists or antagonists (4). When compared to estradiol and estrone, the primary estrogens circulating in women, phytoestrogens are weakly estrogenic (4). In addition, since phytoestrogens come in through the diet, they can be transformed by bacterial enzymes in the intestine, thus affecting their bioavailability (4). In future studies, other parameters of selenium status should be examined relative to soy and FOS intake in order to better understand selenium metabolism in these conditions. In addition, the concentrations of plasma isoflavones should be determined to better understand their relationship to plasma selenium levels.

As soy supplementation continues to be recommended for postmenopausal women and other populations, it is important to understand the effects of its components, including isoflavones, on trace mineral metabolism. The addition of FOS and other supplements that effect gut bacteria, may also affect trace mineral metabolism. Thus, further research is needed to determine how soy and prebiotic supplements affect selenium bioavailability and bioefficacy.

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